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Improved Method of Porcine Pancreas Procurement With Arterial Flush and Ductal Injection Enhances Islet Isolation Outcome

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Abstract

Background—Several pancreas procurement procedures have been used for porcine islet isolation; however, their impact on outcomes has not been extensively studied. We evaluated an advanced procurement technique for porcine islet isolation designed to reduce warm ischemia as well as to remove blood content and enhance cooling of the pancreas by implementing a vascular flush and ductal preservation.

Method—Pancreata procured from adult Landrace pigs were divided into 3 different surgical protocols: Pancreatectomy utilizing only surface cooling (group 1; $n = 24$); surface cooling and ductal injection with cold preservation solution before pancreatectomy (group 2; $n = 12$); or surface cooling, ductal injection, and an approach by selectively flushing through the celiac trunk and the superior mesenteric artery (group 3; $n = 14$). We assessed the islet isolation results and quality using in vitro and in vivo assays.

Results—Significantly higher overall per gram pancreas islet yields were obtained from group 3 pigs compared with the other groups. Measurements of islet viability after 7 days of culture, as assessed by oxygen consumption rate per DNA, showed that group 3 islets displayed the highest values. Sustained normoglycemia was observed in diabetic nude mice transplanted with 2000 islet equivalents from all 3 groups.

Discussion—This study demonstrated that an advanced pancreas procurement technique including ductal preservation and selective arterial flush with cold preservation solution provided significant improvements in porcine islet isolation outcomes.

Intraportal islet transplantation is an emerging treatment option for type 1 diabetic patients experiencing hypoglycemic unawareness. The unlimited and on-demand availability of porcine islets represents unique opportunities for improving the availability and outcomes of cell-based type 1 diabetes therapy.¹ Technical challenges remain, however, to consistently obtain high islet yields from porcine pancreata. Several pancreas procurement procedures have been used for porcine islet isolation; however, their impact on islet isolation outcome has not been extensively investigated. Usually, procurement is done in a slaughterhouse or animal facility without applying typical human surgical procurement techniques. In addition,

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flushing the pancreas with cold preservation solution is not feasible owing to the nonsurgical procurement and logistics related to pig size and location of procurement. It has been demonstrated that warm ischemia of the pancreas of any duration during organ procurement is detrimental to subsequent human islet isolation outcome and functional viability of the islets,² and the blood content of porcine pancreas correlates with a delayed release of islets with greater fragmentation.³ We evaluated an advanced procurement technique for porcine islet isolation designed to reduce warm ischemia, remove blood content, and rapidly cool the pancreas by implementing an arterial flush and ductal preservation approach using cold preservation solution.

Materials and Methods

All experiments were conducted according to the guidelines of the Institutional Animal Care and Use Committee of the University of Minnesota.

Pancreas Procurement and Experimental Protocol

Pancreata procured from adult Landrace pigs after cardiac death were divided into 1 of 3 different surgical protocols: Pancreatectomy utilizing only surface cooling with crushed ice (group 1; $n = 24$); surface cooling and ductal injection with cold preservation solution before pancreatectomy (group 2; $n = 12$); and surface cooling, ductal injection, and a low-pressure vascular flush of cold preservation solution selectively flushing through the celiac trunk and the superior mesenteric artery (group 3; $n = 14$). Cold storage/purification stock solution (Mediatech, Inc., Herndon, Virg), was used as cold preservation solution. Briefly, donor pigs were given an intravenous injection of heparin (250 U/kg), followed by a lethal intravenous injection of sodium pentobarbital 5 minutes later. The pig was exsanguinated by cutting the jugular vein. The pig was then eviscerated by dissecting all connective tissue near the backbone of the pig and the thoracic aorta above the celiac trunk (CT). The viscera were then cooled by crushed sterile ice. The CT and the superior mesenteric artery (SMA) were cannulated selectively. Any vasculature supplied from the SMA or CT that did not perfuse the pancreas is ligated *en bloc*. The pancreas was flushed with 3–4 L of arterial flush solution containing 1000 U/L heparin. Concurrently, the pancreatic duct was cannulated and injected with 60 cc of ductal preservation solution. Pancreatectomy was performed and the pancreas was placed onto a cold pan with cold preservation solution, and dissected free of the surrounding vascular and connective tissue. Intraductal distention with cold (4–8 °C) enzyme solution containing Liberase PI (Roche Molecular Biochemicals, Indianapolis, Ind) was followed. The distended pancreas was transported in cold preservation solution. Warm ischemia time was defined as the time from cardiac death to placement into the cold pan (groups 1 and 2) or the time from cardiac death to start of the arterial flush (group 3). Cold ischemia time was defined as the time from end of warm ischemia to start of pancreas digestion.

Islet Isolation

Pig islets were isolated by the modified Ricordi method.^{4,5} Liberated islets were separated from non-islet tissue on an iodixanol-based (OptiPrep, Axis-Shield, Oslo, Norway) continuous density gradient. The isolated islets were then cultured free-floating in Medium 199 (Mediatech, Inc.) supplemented with 10% pig serum at 37 °C.

Islet Evaluation

The islet yield and purity were assessed by counting duplicate aliquots stained with dithizone before purification, after purification, and after 7 days culture. The number of islets (IC) were converted to islet equivalents (IEQ) with an average diameter of 150 μm .⁶ Fragmentation of islets was estimated by calculating as the ratio between IEQ and IC

number. To assess the potency of isolated islets, oxygen consumption rate per DNA content was measured by using fresh islets and 7-day cultured islets as previously described.^{7,8} To assess islet quality in vivo, the 2000 IEQ fresh islets and 7-day cultured islets obtained from each group were transplanted under the left kidney capsule of diabetic athymic nude mice. Diabetes was induced by intravenous injection of 240 mg/kg streptozotocin (Sigma, St. Louis, Mo). Hyperglycemia was defined as a blood glucose level >400 mg/dL on 2 consecutive days. Normoglycemia was defined when 2 consecutive blood glucose level measured <200 mg/dL. Four weeks after transplantation, nephrectomy of the kidney-bearing graft was performed to demonstrate the return of hyperglycemia.

Statistical Analysis

Data are expressed as mean values $\pm$ standard deviation (SD). Experimental groups were compared using analysis of variance followed by Fisher's PLSD post-hoc test. Comparison of categorical variables was performed with a Fisher's exact test. $P < .05$ was considered significant.

Results

Table 1 shows characteristics of porcine pancreas used for this study in each group. The longest operation time was in group 3; however, the warm ischemic time was shortest in this group. Results of islet isolation are shown in Table 2. Digestion time and islet yield before purification are similar among the 3 groups; however, pellet sizes before purification in group 2 and 3 are significantly larger than that in group 1. Significantly higher purified islet yield was obtained in group 3 ($491,496 \pm 188,184$) IEQ versus $334,850 \pm 114,392$ IEQ for group 1 ($P = .001$); $365,679 \pm 101,339$ IEQ for group 2 ($P = .02$). Islet yield per gram pancreas in group 3 was also significantly higher compared with other groups ($2,209 \pm 863$ IEQ/g vs $1,418 \pm 628$ IEQ/g for group 1 [$P = .001$]; $1,511 \pm 454$ IEQ/g for group 2 [$P = .01$]). Similar levels of islet fragmentation and culture recovery are observed in all groups. Measurements of islet viability after 7 days culture assessed by oxygen consumption rate per DNA showed that islets obtained from group 3 had the highest value among the 3 groups (217 ± 24 vs 180 ± 29 nmol/min $\cdot$ mg DNA for group 1 [$P = .03$]; 210 ± 50 for group 2 [$P = \text{NS}$]). Sustained normoglycemia was observed in diabetic nude mice transplanted with 2000 IEQ from all groups (Fig 1).

Discussion

For porcine islet xenotransplantation to become economically feasible in diabetic patients, it will be desirable to allow restoration of insulin independence with islets isolated from fewer numbers of donor pigs.⁹ An improved porcine islet isolation protocol is required to retrieve consistently high islet yields. This study demonstrates that an advanced pancreas procurement technique, including ductal injection and selective arterial flush with cold preservation solution, can provide significant improvements on porcine islet isolation outcomes. We believe that the technique of porcine pancreas procurement could be among the most important factors for successful islet isolation. We previously demonstrated that selective CT and SMA flush with cold storage solution, in conjunction with ductal infusion, ensures reduction of the core temperature of the pancreas and improves pancreas preservation.¹⁰ Our technique also allows for preservation of the pancreas duct system and removal of pancreas blood content and potential blockages owing to clotting. It has been suggested that these factors are critical to proper distribution and activity of digestion enzymes within the pancreas, and subsequent islet recovery and function.¹⁰ These advancements should be introduced as further development of the porcine islet isolation protocol to ensure successful outcomes in islet xenotransplantation.

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Fig 1.

The cure rate of the transplanted diabetic nude mice with 2000 IEQ at day 0 (**A**) and day 7 (**B**). The cure rate of transplantation using fresh (day 0) islets by 4 weeks after transplantation was 80% in group 1 (standard; $n = 15$), 80% in group 2 (ductal flush; $n = 15$), and 80% in group 3 (ductal/arterial flush; $n = 10$). The cure rate of transplantation using islets cultured for 7 days by 4 weeks after transplantation was 74.1% in group 1 (standard; $n = 27$), 92.9% in group 2 (ductal flush; $n = 14$), and 90.0% in group 3 (ductal/arterial flush; $n = 10$; $P = .89$).

Pancreas Characteristics

Table 1

	Group 1	Group 2	Group 3	P
Pig body weight (kg)	246.8 ± 49.1	254.2 ± 29.5	223.9 ± 33.8	.16
Pancreas weight (g)	242.4 ± 47.0	245.4 ± 33.8	226.4 ± 33.9	.43
Operative time (min)	10.1 ± 2.5	12.4 ± 2.3	15.4 ± 1.2	<.0001
Warm ischemic time (min)	17.0 ± 3.4	20.3 ± 2.3	15.1 ± 1.7	<.0001
Cold ischemic time (min)	69.3 ± 9.9	76.4 ± 8.3	104.1 ± 2.9	<.0001

Analyzed by 1-way ANOVA.

Data are expressed as mean values ± SD.

Table 2
Porcine Islet Isolation Results

	Group 1	Group 2	Group 3	P
Digest time (Phase I)	20.5 ± 3.0	21.2 ± 2.7	21.6 ± 3.5	.59
Before purification IEQ	433 ± 749	422 ± 143	485 ± 172	.59
Before purification IEQ/g	1829 ± 818	1742 ± 594	2083 ± 1101	.56
Pellet size before purification	53.1 ± 23.8	73.5 ± 23.4	74.6 ± 15.0	.01 *
Purified islet yield (×10 ³ IEQ)	335 ± 114	366 ± 101	491 ± 188	.005 *
Purified islet yield (IEQ/g)	1418 ± 628	1511 ± 454	2209 ± 863	.003 *
Isolation index (IEQ/IC)	0.788 ± 0.210	0.679 ± 0.189	0.758 ± 0.199	.34
Purity (%)	87.3 ± 7.1	90.0 ± 7.1	92.0 ± 5.1	.12
7-day culture IEQ recovery	51.8 ± 20.6	63.4 ± 14.0	48.4 ± 13.6	.17
7-day culture DNA recovery	52.4 ± 26.8	45.1 ± 15.8	62.8 ± 16.3	.23
OCR/DNA at day 0	145.9 ± 30.9	130.6 ± 39.2	134.2 ± 17.5	.43
OCR/DNA at day 7	180.1 ± 29.3	210.4 ± 50.2	217.2 ± 24.5	.04 *

Analyzed by 1 way ANOVA; data are expressed as mean values ± SD.

OCR, oxygen consumption rate.

* $P < .05$.